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IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re PATENT APPLICATION of
Ibrahim, et al.

Group Art Unit: 1655

Serial No.: 09/444,095

Examiner: Sisson, B.

Filed: November 22, 1999

FOR: Purification Method and Apparatus

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September 21, 2000

AMENDMENT

Hon. Commissioner of Patents
and Trademarks
Washington, D.C. 20231

Sir:

Responsive to the Office Action dated June 22, 2000, please enter the following amendments and consider the following remarks.

IN THE SPECIFICATION:

Page 12, line 16, replace "they" with ---the---.

IN THE CLAIMS:

Cancel claims 36 and 37.

Cancel non-elected claims 1-30 and 40-62 without prejudice.

Please add claims 63 and 67.

--63. (New) A method of capturing specific DNA or RNA comprising:

placing a purified DNA or RNA sample in a first reservoir tube under conditions to denature double stranded DNA or render RNA suitable for binding;

inserting a wand into said first reservoir tube, wherein said wand comprises a cap, a sample collection assembly and an elongated shaft connecting said cap to said sample collection assembly, said sample collection assembly having microstructures for increasing the surface area of the sample collection assembly, and said sample collection assembly is coated with a sequence specific oligonucleotide probe, peptide nucleic acid

probe through a linker arm, or biotin-streptavidin bond to capture specific target DNA or RNA;

securely and sealingly closing said first reservoir tube with said cap of said wand with said shaft and said sample collection assembly inside said first reservoir tube, and incubating, preferably with agitation, thereby allowing binding of said specific DNA or said specific RNA to said sample collection assembly;

removing said wand from said first reservoir tube and inserting said wand into a second reservoir tube, said second reservoir tube containing a wash buffer;

securely and sealingly closing said second reservoir tube with said cap of said wand with said shaft and said sample collection assembly inside said second reservoir tube;

agitating said second reservoir tube to mix said sample with said wash buffer;

removing said wand from said second reservoir tube and inserting said wand into a third reservoir tube, said third reservoir tube containing an elution buffer;

incubating said third reservoir tube; and

recovering specific DNA or RNA from said third reservoir tube.

64. (New) The method of claim 63, wherein said conditions for denaturing DNA comprise:

adding alkaline buffer, incubating, then adding neutralizing buffer.

65. (New) The method of claim 63, wherein said conditions for denaturing DNA or rendering RNA suitable for binding comprise:

heating said reservoir tube to 95°C for several minutes.

66. (New) The method of claim 63, wherein said microstructures comprise deep reactive ion etchings or toolings that provide a vast surface area on said sample collection assembly.

Cont Sub et E2

67. (New) The method of claim 31, wherein said microstructures comprise deep reactive ion etchings or toolings that provide a vast surface area on said sample collection assembly.---.

REMARKS

Claims 31-35, 37-39 and 63-67 are pending in the application. Non-elected claims 1-30 and 40-62 have been cancelled without prejudice. Applicant reserves the right to refile non-elected claims in a divisional application. Elected Claims 36 and 37 have been cancelled. Claim 36 has been replaced by claim 63. Support for claims 64 and 65 is found in the specification at page 8, second paragraph- page 9. Support for new claims 66 and 67 is found in the specification at page 6, lines 16-17. The specification has been amended to correct a minor typographical error. Now new matter has been added.

Claims 31-36 have been rejected under 35 U.S.C.§112, first paragraph as allegedly not enabled. Applicant respectfully traverses this rejection.

The Examiner states that the claims are enabled for non-specific binding but have the breadth for specific binding and alleges that the specification is not enabled for specific binding. Applicant respectfully disagrees.

The invention addresses purification of sequence-specific applications for example by incorporating singular, or dendritic oligonucleotide or peptide nucleic acid probes to capture specific target molecules. The procedures for capturing sequence-specific nucleic acids using the above molecules are well known in the art (Ausubel F., Brent R., Kingston R.E., Moore D.D., Seidman J.G., Smith J.A., Struhl K., (1987). Current Protocols in Molecular Biology. Greene Publishing Associates and Wiley-Intersciences. John Wiley & Sons, New York, Chichester, Brisbane, Toronto, Singapore; Sambrook J., Fritsch EF, Maniatis J. (1989). Molecular cloning: A laboratory manual. 2nd edition, Cold Spring Harbor laboratory Press, Cold Spring Harbor, New York.). See also page 8, second paragraph for other references incorporated by reference for the capture of nucleic acids, proteins or cells either non-specifically or by affinity binding onto solid phase supports as well as colorimetric, luminescent and fluorescent detection.

The Examiner also states that "there needs to be a denaturation of the target nucleic acid such that it is single stranded prior to hybridization to the probe to the target." Obtaining single-stranded DNA from double-stranded DNA can be achieved by a variety of methods, e.g. alkaline denaturing solutions, exonucleases, or by subjecting the DNA to 95°C temperature for several minutes. These procedures are well known in the state of the art.

The Examiner also states,

> "The specification has been found to contain suggestions that the hybridization step can be conducted subsequent to having performed polymerase chain reaction. Neither the claims nor the specification teach how one is to perform such an amplification reaction when the nucleic acid is present in a protein denaturing solution. Clearly, without something being done to the denaturation solution in which one finds the crude nucleic acid sample, the polymerase used to perform an amplification reaction would be rendered denatured as a direct result of such, be rendered inoperative."

The Applicant respectfully submits that the Examiner has taken the invention out of context. In the description on page 3, second paragraph it states that the present invention in toto, allows much greater flexibility and efficiency and is adaptable to future modification by, for example, incorporating thermal cycling amplification (e.g. PCR), isothermal amplification and fluoregenic, colorimetric, luminescence or electrochemical detection in the same device. It does not suggest that the polymerase chain reaction can be performed in the presence of a protein denaturing solution. Such a basic fact is well known to any person with ordinary skill in the art. PCR amplification is not the subject of the claims 31-35 or 36. Therefore, the claims 31-35 are enabled by the current specification and could be practiced by one of ordinary skill in the art.

Claims 31-36 have been rejected under 35 U.S.C. §112, second paragraph as allegedly incomplete for omitting essential steps under MPEP 2172.01. Applicant respectfully traverses this rejection.

MPEP 2172.01 states that "essential matter may include missing elements, steps or necessary structural cooperative relationships of elements described by the Applicant as necessary to practice the invention." Applicant submits that no essential steps are missing that are described by the applicant as necessary to practice the invention.

The alleged omitted steps are:

a. How the nucleic acid is to be prepared such that it is used in a hybridization reaction;
b. If one is to conduct an amplification reaction, just how the amplification reaction is to be performed while there is a protein denaturant present (seemingly, the protein denaturant will result in rendering the polymerase inoperative). If one is to use a peptide probe after amplification, and the peptide probe is anti-histone antibody, the method needs to reflect what steps are needed so to render the amplified nucleic acid capable of being bound by the antibody, if such is even possible; and
c. Like "b", supra, the use of a peptide probe, e.g., an antibody, to bind the nucleic acid, while the protein denaturant is present, raises several issues. The claims need to reflect just what steps are needed so to render the sample mixture susceptible to binding by another protein when the presence of denaturant would result in the denaturation of the peptide probe.

Regarding a. above, a hybridization reaction is not the subject matter of claims 31-35. Thus, according to MPEP 2172.01, it is not necessary for the practice of the invention as set forth in these claims. However, a hybridization reaction would only be relevant for new claim 63 if sequence-specific nucleic acids purification is desired. Such a procedure is described in Example 2 on page 12. In such a case, the capture assembly is coated with oligonucleotide or peptide nucleic acid probes of know sequence by using methods that are known in the art. The oligonucleotide probes can be used to capture rRNA, mRNA, total RNA, or single-stranded DNA molecules by using methods that are known in the art.

Regarding b. above, no suggestion was made to perform amplification reactions in the presence of denaturant in claims 31-35 or 63 or anywhere else in the invention. It was suggested that the invention is adaptable to future modification by, for example, incorporating thermal cycling amplification (.e.g. PCR), isothermal amplification and fluorogenic, colorimetric, luminescence or electrochemical detection in the same device. The description of the future adaptations is not the subject of claims 31- 35 or 63.

Further, using a peptide probe or anti-histone antibody after amplification is not the subject of claims 31-35 or 63.

Regarding c. above, claim 63 states that the purified DNA or RNA sample is placed under conditions for binding the specific DNA or RNA to the sample collection

assembly. One of ordinary skill in the art would readily understand that what these conditions would be in light of the what is known in the art and the articles disclosed on page 8 of the application.

Thus, claims 31-35 are in compliance with 35 U.S.C.§112. second paragraph.

Claims 31-39 have been rejected under 35 U.S.C. §103(a) as allegedly unpatentable over Ji et al., in view of Henco et al., Piaso et al., Lockhart et al., and Tuunanenn. Applicant respectfully traverses this rejection.

The present invention relates to a method that employs an apparatus for the purification and concentration of nucleic acids such as DNA or RNA from a sample without the need for centrifugation, precipitation, lengthy incubations, magnetic fields or gravity. The sample may be blood or urine, for example. The method of the invention uses a series of reservoir tubes and a wand. The wand has a sample collection assembly with microstructures that provides vast increase in surface area for collecting greater quantities of nucleic acids. The sample is placed in a first reservoir tube with a sample lysis/denaturing solution wherein the nucleic acids are released from cells and are available for binding to the sample collection assembly in a first tube. After the nucleic acids bind to the sample collection assembly, the wand is then placed in a second reservoir tube with a wash buffer to wash the nucleic acids and then the wand is placed in a third reservoir tube containing an elution buffer where it is incubated and the purified DNA or RNA is recovered.

The employment of the wand with microstructures on its sample collection assembly and the series of tubes permits a simple method for DNA or RNA purification. This method is useful and convenient, particularly for field usage in remote locations such as in times of war or contingency operations where centrifuges or other intricate and complicated equipment are difficult to operate and impractical to carry.

Additionally, the invention is directed to recovering specific DNA or RNA from a purified sample of DNA or RNA using the reservoir tubes and wand of the invention as set forth in claim 63. None of the cited references disclose the method of the invention.

Ji et al. is directed to a triplex-mediated capture method of isolation of specific target tract from circular bacterial DNA using specific oligonucleotide sequence and solid

support as magnetic beads. The present invention is different in scope and content since it can purify nucleic acids from a sample without knowing the target sequence. (However, the apparatus and methods can also be used for isolation of specific nucleic acids targets by using oligonucleotide or peptide nucleic acids probes). In addition, the present invention describes a capture assembly that contains a wand and microstructures. The use of this capture assembly does away with the need for a magnetic field, filter paper, or columns (which requires gravitational or pressure forces) as required by Ji's invention. Therefore, would not have lead one of ordinary skill to the present invention.

Henco et al. does not make up for the deficiencies of Ji et al. Henco et al. discloses a device comprising porous matrix bed in a column, which contains silica gel or teflon particles for immobilizing the cells that are to be lysed. The use of such device requires pre-processing of the samples outside of the device for aggregation. (see column 4, lines 49-53). The process described by Henco et al. for immobilizing, washing and elution of DNA require gravitational or pressure forces, e.g., precipitation or centrifugation (col. 4, lines 53-57). In the present invention the sample is processed in the device itself (the reservoir) without the need of centrifugation and does not require gravitational or pressure forces for washing or elution of the DNA. The present invention also does not require cell immobilization for fixing DNA through a column or column matrix. Therefore, Henco, et al. is much more complicated than the presently claimed device, which does not require centrifugation or pressure forces. Hence, Henco et al. does not make up for the deficiencies of Ji et al.

Piasio et al. describe a method and apparatus for conducting a chemical reaction, primarily of antigen-antibody nature. Piasio et al. does not teach the use of the apparatus for purification of nucleic acids. Piasio et al. discloses a variety of solid support shapes that consist of binding member having a smooth plane or curved surface. Piasio et al. actually teaches away from the present invention in this regard, stating *"The essential features of the matrix include a handle member and a plurality of essentially smooth plane or curved surfaces attached thereto..."* The present invention does not use the smooth surface essential to Piasio et al. The present invention claims microstructures on the sample collection assembly that can be etched and cross-etched lanes, dimples,

pillars, pores or microparticles (which provide vast surface area for far more efficient binding). Therefore, one of ordinary skill in the art would not be motivated to use the wand in Piasio et al. with the methods in Ji et al. or Henco et al.

Lockhart et al. describe surface-bound, unimolecular, double-stranded DNA relating to the field of polymer synthesis and double-stranded oligonucleotide library screening. Lockhart et al. does not teach devices and methods for purification of nucleic acids. Further, Lockhart et al. does not describe any components similar to the capture assembly of the invention, nor do they describe the use of microstructures. Rather, Lockhart et al. teaches that the solid support is preferably flat, which teaches away from the invention. This is because Lockhart et al. is not concerned with maximizing binding of DNA in a sample by increasing surface area. Thus, Lockhart et al. does not provide the necessary motivation to modify Ji et al., Henco et al. or Piaso et al. to arrive at the present invention.

Tuunanen (WO 94/18564) discloses equipment and methods specifically for performing solid-phase immunoassays. The solid-phase support described by Tuunanen does not include microstructures or specific nucleic acids binding material. The increase in surface area for immunoassays are created by relatively large protrusions, cavities or grooves. This is not comparable to the vast increase of surface area created by microstructures via deep reactive ion etching or microparticles presently claimed.

Unlike, the microstructures of the present invention, the grooves in Tuunanen were specifically described as leading downward to the point to for easier fluid flow off the body for the multiple washings. The multiple washing description of Tuunanen relates specifically to the washing of an immunocomplex, not nucleic acids. Therefore, Tuunanen does not provide a teaching or suggestion for employing microstructures on a wand to be used in a method of DNA or RNA purification to make up for the deficiencies of the above discussed references.

None of the cited references whether taken alone or in combination would have lead one of ordinary skill in the art to the present invention because none of them provide a method for DNA or RNA purification that employs a wand having a sample collection assembly with microstructures and a series of reservoirs for processing a sample and

ultimately retrieving the purified DNA. Therefore, the rejection under 35 U.S.C. §103(a) is believed overcome.

Reconsideration and allowance are respectfully requested. The Examiner is invited to telephone Applicant's representative at (301) 924-9500 if it would in any way expedite prosecution.

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Respectfully submitted,
By: Caroline Nash

Caroline Nash, Reg. No. 36, 329
Nash & Titus, LLC
3415 Brookeville Rd., Suite 1000
Brookeville, MD 20833

Elizabeth Arwine, Reg. No. 45,867
U.S. Army Medical Research and
Materiel Command,
Attn: MCMR-JA,
Fort Detrick, MD 21702-5012